

XP-002306643

Dp

(C) WPI/Derwent

AN - 1997-186935 [17]

AP - JP19960153043 19960524; [Previous Publ. JP9047229]

CPY - OMUN-N

DC - D13 D16

DR - 0009-S 0419-S

FS - CPI

IC - A23J3/08 ; A23J3/34 ; A61K35/20 ; A61K38/00 ; A61K38/17 ; A61P37/08 ;
C12P21/06

MC - D03-B01 D03-H01T2 D05-A02C D05-C03C

PA - (OMUN-N) OMU NYUGYO KK

PN - JP3542093B2 B2 20040714 DW200446 A23J3/08 010pp

- JP9047229 A 19970218 DW199717 A23J3/08 007pp

PR - JP19950159888 19950601

XA - C1997-059631

XIC - A23J-003/08 ; A23J-003/34 ; A61K-035/20 ; A61K-038/00 ; A61K-038/17 ;
A61P-037/08 ; C12P-021/06

AB - JP09047229 A protease-hydrolysed conc milk compsn is new. Also claimed
is the prodn of the compsn.

- A milk compsn having a concn higher than before hydrolysis by 80 mOsm
or more as determined by the freezing pt lowering method is new. The
prodn comprises hydrolysing milk protein with proteases, thereby
raising the concn of the compsn by 80 mOsm or more, as determined by
the freezing pt lowering method, than before hydrolysis. The prodn
pref comprises adjusting the pH of the hydrolytic prod to acidic; or
lactic acid-fermenting the hydrolytic decomposate, thereby adjusting
the pH to 4.5 or less.

- USE/ADVANTAGE - This milk compsn is functional peptide food. This milk
compsn with its concn raised by protein hydrolysis has its bitterness
and allergenicity suppressed. Hydrolysis with proteases is applicable
to any form of liq. Milk or liq milk prod e.g. fresh, defatted,
processed, or concn milk, liquefied whey, etc. For proteases, alkalase
(Bacillus licheniformis-derived end-type protease, Novo Nordisk)
and/or flavourzyme should pref be used. The reaction temp and time
depend on the enzyme used. With alkalase and/or flavourzyme,
hydrolysis proceeds at 50-55 deg C for 2-6 hours. The concn of the
decomposate is measured in the course of hydrolysis using an
osmometer. The pH adjustment may be done by adding appropriate acids,
eg lactic acid, citric acid, etc, or pref lactic acid-fermenting the
hydrolytic prod.

- (Dwg.0/6)

IW - BITTER SUPPRESS MILK COMPOSITION PRODUCE HYDROLYSIS MILK PROTEASE

IKW - BITTER SUPPRESS MILK COMPOSITION PRODUCE HYDROLYSIS MILK PROTEASE

NC - 001

OPD - 1995-06-01

ORD - 1997-02-18

PAW - (OMUN-N) OMU NYUGYO KK

TI - Bitterness- and allergenicity-suppressed milk compsn. - produced by
hydrolysis of milk by protease.

D-12

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CLAIMS

[Claim(s)]

[Claim 1] The milk constituent which is a milk constituent obtained by hydrolyzing milk protein with proteolytic enzyme, and is characterized by the concentration by the freezing-point-depression measuring method being [more than 80 (mOsm)] larger than hydrolysis before.

[Claim 2] The manufacture approach of the milk constituent characterized by enlarging milk protein more than 80 (mOsm) including the process hydrolyzed using protease rather than concentration by freezing-point-depression measuring method hydrolysis-before.

[Claim 3] The manufacture approach of the milk constituent of claim 2 characterized by including the process which adjusts pH of a hydrolysis product to acidity.

[Claim 4] The manufacture approach of the milk constituent of claim 3 characterized by adjusting pH to 4.5 or less by carrying out lactic acid fermentation of the decomposition product after hydrolysis after hydrolyzing using protease and enlarging milk protein more than 80 (mOsm) rather than concentration by freezing-point-depression measuring method hydrolysis-before.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] Few moreover, especially as for this invention, bitterness is related with an allergenic low milk constituent and its allergenic manufacture approach about a milk constituent.

[0002]

[Description of the Prior Art] Various kinds of milk constituents originating in cow's milk etc. are used for the raw material of special nutritive foods as food or a functional peptide constituent as they are.

[0003] The problem in such dairy products is a food allergy, and being absorbed by the inside of the body while this had left antigenic, without fully disassembling protein, such as cow's milk which is a foreign protein for Homo sapiens, is considered as the cause. The means currently present most generally performed for the purpose of obtaining a functional peptide further for the purpose of reducing whether it abolishes allergenic [of such dairy products] is the enzymatic hydrolysis of milk protein.

[0004] However, it has been the technical problem which must be solved when bitterness will arise and it will use a milk protein decomposition product as food or a food functional raw material, if protein is generally disassembled. The peptide of various sizes generates this by decomposition, and it is considered for the hydrophobic side chain which existed in the interior of proteinic till then to be exposed.

[0005] many of approaches from the former for obtaining a milk constituent by zymolysis for the purpose of the reduction in the allergen of milk protein use that to which the component was adjusted beforehand as a substrate (milk protein raw material) of an enzyme, or it mixes the decomposition product of (JP,4-320650,A) or casein, or milk serum protein later -- etc. (JP,5-17368,A) etc. -- a special raw material is used.

[0006] It is desirable to make the whole milk protein into a substrate from the point of a nutrition (in amino acid composition, the content of casein of the cystine which is sulfur containing amino acid is remarkably low.). since the cystine is considerably contained in the whey protein, if the whole milk is used -- nutritional -- a problem -- it is not -- such a technique is not found. Moreover, also when no these approaches are devised especially about the dissolution of bitterness and it is not suitable as food or a food functional raw material, a certain thing is clear.

[0007] The low allergenic peptide constituent which has oral tolerance induction ability is obtained and ****(ed) by JP,5-5000,A by understanding the protein of the cow's milk origin by the enzyme, and making molecular weight or less into 10,000. However, according to the knowledge which this invention person found out, from 10,000, molecular weight is made low for how many minutes, it orders molecular weight, and then sufficient antigenic lowering is not obtained, and bitterness cannot be canceled, either.

[0008] How to make bitter peptides hydrolyze further by exopeptidase as a bitterness removal method (For example) KM.Cleggand A.D.Mc Millian J.Food Techonol. 9:21 (1974) ; H.Umetsu H.Matsuoka and E.Ichishima J.Agric.FoodChem. 31 : 50 (1983) Although used By this approach, flavor is spoiled extremely. The method of stopping extent of zymolysis, in order to suppress generation of II which bitterness tends to generate in addition to this in order to remove bitterness, or tripeptide, or removing a low-molecular peptide using the film is also taken. However, antigenic

lowering sufficient by such technique is not obtained.

[0009]

[Means for Solving the Problem] this invention person came to draw header this invention for the concentration by the freezing-point-depression measuring method correlating milk protein with the bitterness of a milk constituent, and allergenic as physical properties of the milk constituent obtained by hydrolyzing, as a result of repeating research, in order to solve the above problems.

[0010] In this way, this invention is a milk constituent obtained by hydrolyzing milk protein with proteolytic enzyme, and the bitterness characterized by the concentration by the freezing-point-depression measuring method being [more than 80 (mOsm)] larger than hydrolysis before offers a low allergenic milk constituent few.

[0011] Moreover, this invention is an approach for bitterness to manufacture few [and] low allergenic milk constituents as another view, and offers the approach characterized by enlarging milk protein more than 80 (mOsm) including the process hydrolyzed using protease rather than concentration by freezing-point-depression measuring method hydrolysis-before. In a desirable mode, the approach of this invention includes adjusting pH of a hydrolysis product to acidity. And if the most desirable mode of this invention is followed, after hydrolyzing using protease and enlarging milk protein more than 80 (mOsm) from concentration by freezing-point-depression measuring method hydrolysis-before, pH is adjusted to 4.5 or less by carrying out lactic acid fermentation of the hydrolysis product.

[0012]

[Embodiment of the Invention] The description of the milk constituent of this invention is to specify the difference with the value before hydrolysis about the concentration by the freezing-point-depression measuring method.

[0013] Thus, the concentration by the freezing-point-depression measuring method used about this invention is expressed by OZUMORU (OZUMORARITI, Osmolarity) of the solute molecule in 1kg of pure water as follows.

$$\text{OZUMORARITI} = \text{Osm (OZUMORU)} / \text{kg H}_2\text{O} = \phi n c$$
 -- here, an osmotic coefficient (extent of dissociation of a molecule is expressed) dissociates ϕ , a molecule dissociates n , and the number of the made particles and c show the mol concentration of a solution.

[0014] Although the freezing point will descend if a solute is dissolved in a pure solvent as known well, this is considered for the affinity or the condensation property of a solvent to change in proportion to the concentration of a solute. Therefore, the concentration (the number of the particles in a solution) of a solute can be known by performing freezing-point-depression measurement. One mol of matter dissociated thoroughly ideally is not actually dissociated thoroughly, although the freezing point of pure water is reduced by 1.86 degrees C. It is because dissociation decreases according to the factor to which interference between solute molecules is called an osmotic coefficient (ϕ).

[0015] In a water solution, matter 1mOsm (milli OZUMORU = 10^{-3} Osm) carries out 1.86m degree-C lowering of the freezing point to 1kg of water. That is, it is thought that the unit of mOsm or Osm is reflecting concentration or the number of mols of a solute which contributes to freezing point depression among the solutes which exist in a solution.

[0016] In order to perform the cryoscopy of a solution to accuracy promptly, after carrying out abundance supercooling of the freezing point of the solution further, it is made to freeze mechanically and the temperature is measured. The temperature of the solute emitted rapidly is raised to the equilibrium (plateau) of water and ice (sherbet condition). This equilibrium state is searched for for OZUMORARITI as the freezing point of a solution. Such measurement is performed using OZUMO meter.

[0017] In a milk constituent, the difference of the reason correlated with the bitterness of the milk constituent concerned or allergenic with the value before hydrolysis of the concentration by such freezing-point-depression measuring method is not yet completely clear. In a milk constituent which is made into the object of this invention, concentration which is expressed with OZUMORARITI may have contributed as synthetic concentration which, on the whole, displays the amount of the component which participates in bitterness, such as protein, sugar (lactose etc.), and mineral, or antigenic.

[0018] This invention can face and apply the dairy products of which type which contains the milk protein originating in cow's milk etc. as a raw material (substrate) for carrying out enzymatic hydrolysis. Although generally applied to the milk constituent obtained by carrying out enzymatic hydrolysis of the liquefied milk, such as fresh milk, cow's milk (usually cow's milk), a skimmilk, liquid milk containing recombined milk, concentrated milk, and various milk beverages, it is similarly applied to the liquefied object which remelts or suspends and can obtain the dairy products of the shape of powder, the shape of a solid, and jelly. For example, this invention is applied also to the milk constituent obtained [use / as a raw material / what liquefied whey powder] by carrying out enzymatic hydrolysis.

[0019] Namely, with the milk constituent of this invention, it originates in cow's milk etc. and is obtained by presenting enzymatic hydrolysis with casein, beta-lactoglobulin, alpha-lactalbumin, and dairy products that generally have a total protein content to 2 - 10% of the weight as a liquefied object by containing all or the parts of amino acid etc., such as a cystine besides protein, such as an immunoglobulin and serum albumin, further.

[0020] Although various kinds of enzymes can be theoretically used for hydrolyzing milk protein according to this invention, this invention persons have found out that what is called the enzyme, for example, the alcalase, and flavor ZAIMU of the microorganism origin is excellent.

[0021] alcalase is sold from Novo Nordisk (Novo Nordisk) -- having -- *Bacillus licheniformis* from - it is the end mold protease obtained. The main enzyme component is the subtee lysine A (Subtilisin Carlsberg), and an active center is a serine. Moreover, flavor ZAIMU is too sold from Novo Nordisk (NovoNordisk). *Aspergillus oryzae* It is multienzyme which has both the activity of the end mold protease of the origin, and an exomold protease. Although each may be independently used for these enzymes, the outstanding effectiveness is acquired when both are mixed and used.

[0022] The reaction temperature and reaction time in a hydrolysis reaction by the enzyme change a little with enzymes to be used. In the alcalase which is a suitable enzyme to enforce the approach of this invention, or flavor ZAIMU, generally, a hydrolysis reaction is performed in 50-55 degrees C for 2 to 6 hours. Under the present circumstances, according to progress of a hydrolysis reaction, it samples at suitable spacing, and the concentration by the freezing-point-depression measuring method is measured, checking deactivation extent of an enzyme. In order to check allergenic [of a milk constituent], the determination of molecular weight by liquid chromatography has been performed from before. However, the freezing-point-depression measurement in OZUMO meter [as / in this invention] is remarkably simpler than the determination of molecular weight by liquid chromatography, and the approach of this invention is advantageous also in this point.

[0023] this invention person's header -- according to the data, on the occasion of manufacture of a milk constituent including such an enzymatic hydrolysis process, a low allergenic milk constituent with quite little bitterness is preferably obtained by enlarging more than 90 (mOsm) more than 80 (mOsm) at least rather than concentration by freezing-point-depression measuring method hydrolysis-before the bottom. If the desirable mode of this invention is followed at this time, it is common to hydrolyze milk protein using protease and to enlarge concentration by the freezing-point-depression measuring method more than 80 (mOsm). However, while enlarging concentration by the freezing-point-depression measuring method to some extent by enzymatic hydrolysis, it can also enlarge more than 80 (mOsm) from concentration by freezing-point-depression measuring method hydrolysis-before with combination with the process before and behind that (for example, pH adjustment process or the decomposition process using the enzyme of another kind further).

[0024] Moreover, if this invention is followed, a low allergenic (antigenic) milk constituent especially with little bitterness can be obtained by adjusting pH of the decomposition product after hydrolysis to an acid field. Generally pH is adjusted to 4.5 or less, and when it is desirable to be referred to especially as 4.0-4.5 and pH becomes low from this, it has the inclination for an acid taste to become strong.

[0025] Adjustment of pH is theoretically possible also by adding the only suitable acid (for example, a lactic acid, a citric acid) for the constituent after hydrolysis. However, if the desirable mode of this invention is followed especially, pH will be adjusted to 4.5 or less by performing lactic acid fermentation using lactic acid bacteria. Thereby, it becomes mellower as a flavor and an acid taste adjust from an acid, the improvement of the flavor by fermentation is obtained, or the immunity

activation operation and antibacterial action by lactic acid bacteria (biomass component) are obtained, and also desirable effectiveness is given to absorption of calcium or an advantage, like there is a lowering operation of cholesterol is added. Especially the lactic acid bacteria used for lactic acid fermentation are not limited, and, generally are easy to be used. After making it ferment until it adds lactic acid bacteria and is set to predetermined pH, it is checked that heat-sterilized (it heats for 30 minutes at 65 degrees C preferably), fermentation was suspended, the concentration by the freezing-point-depression measuring method was measured, and the desired milk constituent has been obtained.

[0026] If this invention is followed as mentioned above, though it is low allene gene nature, the milk constituent with which there is little bitterness and flavor is not spoiled, either will be obtained. In addition, when it measures with liquid chromatography, a part into which, as for the milk constituent of this invention, molecular weight exceeds 5000 does not exist substantially, but it is checked that especially the with a molecular weight of 1000 or less thing occupies the part (80% or more) considerably. Moreover, the content of the free amino acid on the basis of the whole milk constituent is about 20 or less % of the weight per amount of total nitrogen. The milk constituent of this invention can be used also as the raw material same as pre-mixed powder when making a pan and confectionery etc. as the usual milk powder (depowder, all powder) by being able to use it further, in case jelly, ice cream, a cream, a milk beverage, etc. are prepared as a food raw material, and making it dry, although it can offer as a drink as it is.

[0027]

[Example] This invention is not limited by these examples, although this invention is hereafter explained in accordance with an example in order to clarify the description of this invention further.

[0028] [Example 1]

As an enzymatic hydrolysis reaction raw material, casein, beta-lactoglobulin, alpha-lactalbumin, the lactose, and the fat were contained and cow's milk (usually cow's milk) of the 3 % of the weight of the amounts of total protein was used. It was 285 (mOsm) when the concentration by the freezing-point-depression measuring method of this cow's milk was measured. Temperature up of this was carried out to 50 degrees C, and alcalase 0.02% (v/v) and flavor ZAIMU 0.01% (w/v) were added to this. After enzyme addition and after having performed enzymatic hydrolysis, having sampled for every hour, heating for 20 minutes at 90 degrees C and carrying out deactivation of the enzyme, stirring at 50 degrees C, concentration by the freezing-point-depression measuring method was measured. The enzymatic hydrolysis reaction was performed till 6 hours. In addition, measurement of the concentration by the freezing-point-depression measuring method is FISKE. It carried out using the OZUMO meter mark 3 of a shrine.

[0029] Lactic acid fermentation was presented with the milk protein after hydrolysis by the lactic-acid-fermentation enzyme, and pH was adjusted. That is, it fermented after carrying out inoculation of the lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) which carried out preculture with the decomposition skim milk beforehand 1.5% (v/v) until it was set to pH4.5 at 37 degrees C. Then, it heated at 65 degrees C for 30 minutes, and fermentation was stopped.

[0030] The assessment trial of bitterness was carried out about the decomposition fermented milk obtained by the decomposition milk and lactic acid fermentation which were got by the above-mentioned enzymatic hydrolysis by ten persons' panelist by whom bitterness assessment test training was done. That is, mark were given, having set to 5 the bitterest thing in the decomposition milk sampled for every time amount, and decomposition fermented milk during the reaction of 1 - 6 hours, and having used as 0 the case where bitterness was not sensed. The result about decomposition milk (before lactic acid fermentation) is shown in the graph of drawing 1. Among the graph, an axis of ordinate shows the sum total of the point evaluating [bitterness], and an axis of abscissa shows the value of the difference which subtracted the concentration by the freezing-point-depression measuring method before decomposition from the concentration by the freezing-point-depression measuring method of each decomposition milk, and among the graph, it expresses the data after the reactivity in every hour as it goes to the right from the left. Although not shown in drawing, the points of decomposition fermented milk of having presented the above-mentioned lactic acid fermentation with the enzymatic hydrolysis reaction 3 hours, 4 hours, 5 hours, and after carrying out for 6 hours totaling [bitterness assessment] were 21, 17, 15, and 15, respectively. In addition,

the increment of the concentration by the freezing-point-depression measuring method of these decomposition fermented milk in [-10 (mOsm)] is carried out more slightly than the value after an enzymatic hydrolysis reaction, therefore its all are [more than 80 (mOsm)] larger than hydrolysis before.

[0031] Bitterness increases with the increment in the concentration by the freezing-point-depression measuring method so that I may be understood from drawing, but if a difference with the concentration by the freezing-point-depression measuring method before decomposition exceeds about 80 (mOsm), it will decrease, and if especially 90 (mOsm) is exceeded, it will decrease sharply. And the bitterness of a milk constituent is further mitigated by carrying out lactic acid fermentation to the decomposition product with which the difference exceeded 80 (mOsm), and the decomposition product exceeding especially 90 (mOsm).

[0032] Measurement of the molecular weight of the milk constituent (that with which lactic acid fermentation was presented after the enzymatic hydrolysis of 6 hours) which is beyond measurement of molecular weight, and was made and obtained was tried. Measurement was performed using liquid chromatography and TSK-GELG2000SWXL (TOSOH CORP. make) was used as a column. The sample dissolved freeze-drying powder in the mobile phase by the concentration of 2mg/ml, and filtered it with the 0.45-micrometer filter.

[0033] 45% acetonitrile which contains TFA 0.1% was used for the mobile phase. Measurement was performed at the room temperature and the absorbance in 215nm was detected, using an ultraviolet absorptiometer as 0.2ml a part for /and the detector of the rates of flow. They are Cytochrome C (MW:12,500), an insulin (MW:5749.5), and an insulin as a marker of molecular weight. chain BFragment Using 22 -30 (MW:1086.3) tryptophan (MW:204.23), the molecular-weight-distribution calibration curve was created and the elution time amount of molecular weight 10, 000, 5,000, and 1,000 was found.

[0034] The result is shown in I of drawing 2 . As shown in drawing, the part exceeding molecular weight 5000 does not exist in the milk constituent of this invention. And the with a molecular weight of 1000 or less part occupies most (about 80%).

[0035] It measured as follows an antigenic test and antigenic [which were acquired / of a milk constituent] by control ELISA trial. The used approach is the control ELISA trial by Rat IgG. An antigen solution (1mg/(ml)) 100microl impregnation to each well After carrying out and fixing by leaving it at 37 degrees C for 1 hour, It is 0.15M about a well. NaCl and the phosphate buffer solution which contains Tween20 0.05% (pH7.2) It washes 3 times with (the following, PBS-Tween, and a brief sketch), and is PBS-Tween. 2.0 dissolved% Fish Gelatin 300microl impregnation of a solution was done and it blocked by leaving it overnight at 4 degrees C.

[0036] Subsequently, PBS-Tween After 3 times washing, 100micro (what mixed 100micro of decomposition fermentation objects I which carried out phase dilution with the phosphate buffer solution (pH7.2) (the following, PBS, and brief sketch) containing 0/15M NaCl, and 100micro of anti-skimmilk antisera I of the rat diluted 1000 times, and was left at 4 degrees C overnight) of blood serum-sample mixture I was poured in, and it was left at 37 degrees C for 1 hour. Secondary antibody which furthermore carried out the indicator of the peroxidase after washing a well Fish Gelatin It diluted with the solution 1000 times, it poured in every [100micro / 1], and was left at 37 degrees C for 1 hour. Poured in 100micro of substrate solutions I, it was made to react after washing a well for about 15 minutes, 100micro of oxalic acid solutions I was added 1.5%, and the reaction was stopped. Measurement is Immuno. It carried out by 405nm using ReaderNJ-2001 (Inter Med company make), and asked for the rate of control according to the following formula.
Rate (%) of control = $(A_0 - A) / (A_0) \times 100$, however A are the absorbances at the time of making a blood serum and a sample react, and A₀ is an absorbance at the time of making a blood serum and PBS react.

[0037] Consequently, that with which lactic acid fermentation was presented after the enzymatic hydrolysis of 6 hours is shown in II of drawing 2 . Although it is raw material cow's milk which white shows and it is the milk constituent sample which black shows, for antigenic, cow's milk is 105 about 1/. Falling is understood. In addition, when the amino-acid-analysis machine analyzed the free amino acid content of the obtained milk constituent, it was 14 % of the weight per amount of total nitrogen. The result with the same said of other milk constituents was obtained.

[0038] If it removed having carried out addition of the alcalase 0.02% (v/v) independently as a [example 2] enzyme, enzymatic hydrolysis reaction and lactic acid fermentation were performed like the example 1. When the bitterness assessment trial was performed measuring concentration by the freezing-point-depression measuring method like an example 1 on the occasion of an enzymatic hydrolysis reaction, a result like drawing 3 was obtained. Moreover, the values of the point of decomposition fermented milk of having presented lactic acid fermentation with the enzymatic hydrolysis reaction 5 hours and after carrying out for 6 hours totaling [bitterness assessment] were 30 and 27, respectively. In addition, when the concentration by the freezing-point-depression measuring method was measured about these decomposition fermented milk, it was increasing more slightly than the value after enzymatic hydrolysis, and it was found out that all are [more than 80 (mOsm)] larger than hydrolysis before. If the difference of the concentration by the freezing-point-depression measuring method exceeds 80 (mOsm) so that I may be understood from these results, bitterness will be reduced, and the effectiveness will increase further, if it passes through lactic acid fermentation.

[0039] moreover, the place which carried out the control ELISA trial like the case of an example 1 about the obtained milk constituent (decomposition fermented milk) -- antigenic -- about [of raw material cow's milk] -- 1/104 Decreasing was found out.

[0040] If it removed having carried out addition of flavor ZAIMU 0.01% (w/v) independently as a [example 3] enzyme, enzymatic hydrolysis reaction and lactic acid fermentation were performed like the example 1. On the occasion of the enzymatic hydrolysis reaction, the bitterness assessment trial was performed like the example 1, and the result of drawing 4 was obtained. In addition, the values of the point of decomposition fermented milk of having presented lactic acid fermentation with the enzymatic hydrolysis reaction, and having obtained it 5 hours and after carrying out for 6 hours totaling [bitterness assessment] were 30 and 28, respectively. Moreover, the concentration by the freezing-point-depression measuring method of these decomposition fermented milk is increasing more slightly than the value after enzymatic hydrolysis, and is [more than 80 (mOsm)] larger than hydrolysis before. If the difference of the concentration by the freezing-point-depression measuring method exceeds 80 (mOsm) so that I may be understood from these results, bitterness will decrease, and the decomposition fermented milk especially by lactic acid fermentation shows remarkable bitterness reduction.

[0041] moreover, the place which measured antigenic by the control ELISA trial shown in the example 1 about the obtained milk constituent (decomposition fermented milk) -- raw material cow's milk -- comparing -- about -- 1/104 It was falling.

[0042] When enzymatic hydrolysis reaction and lactic acid fermentation were performed like the example 1 and the bitterness assessment trial was carried out as a [example 4] raw material using the liquefied object which melted in water the whey powder which contains beta-lactoglobulin, alpha-lactalbumin, and a lactose as a principal component so that the amount of total protein might become 3.1 % of the weight, the result of I of drawing 5 was obtained about the enzymatic hydrolysis product. Moreover, the values of the point of a decomposition fermentation object of having presented lactic acid fermentation with the enzymatic hydrolysis reaction 4 hours, 5 hours, and after carrying out for 6 hours totaling [bitterness assessment] were 20, 20, and 18, respectively. In addition, the concentration by the freezing-point-depression measuring method of these decomposition fermentation objects is increasing more slightly than the enzymatic hydrolysis back, and its all are [more than 80 (mOsm)] larger than hydrolysis before. If the concentration by the freezing-point-depression measuring method exceeds 80 (mOsm) also in this case, bitterness will decrease, so that I may be understood from these results, and if it passes through lactic acid fermentation, bitterness will be reduced further.

[0043] Moreover, when the control ELISA trial was carried out like the case of an example 1 about this whey liquefied object, antigenic is the abbreviation 1/104 of the whey liquefied object of a raw material. It was decreasing (refer to II of drawing 5).

[0044] Enzymatic hydrolysis was performed like the example 1, having carried out addition of the trypsin 0.004% (w/v) as a [example of comparison] enzyme, and having used reaction temperature of enzymatic hydrolysis as 37 degrees C. When the bitterness assessment trial was performed measuring the concentration by the freezing-point-depression measuring method like an example 1

about what was serially sampled according to progress of an enzymatic hydrolysis reaction, the result of drawing 6 was obtained. As shown in drawing, the difference with the concentration by the freezing-point-depression measuring method before the hydrolysis after reaction (6 hours) termination is about 70 (mOsm), and having remained remarkable bitterness was admitted. In addition, pH of an enzymatic hydrolysis resultant was 5.5. Moreover, when the control ELISA trial same about a resultant as an example 1 was performed, antigenic lowering was only about 1 of raw material cow's milk/10.

[Translation done.]

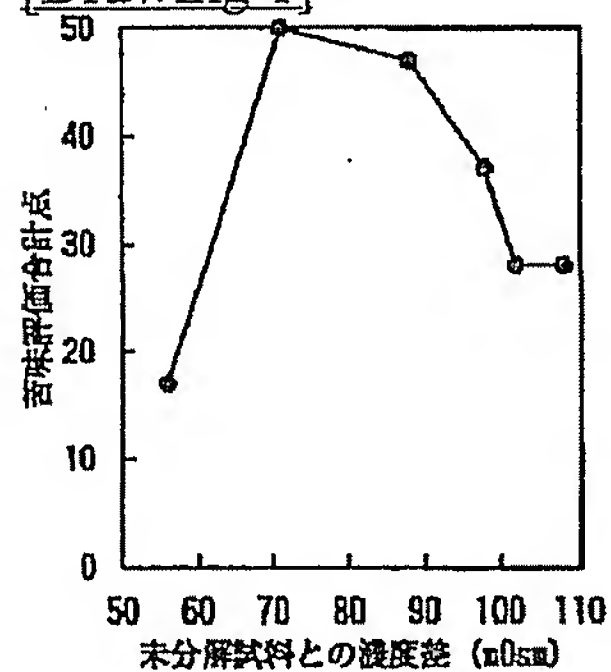
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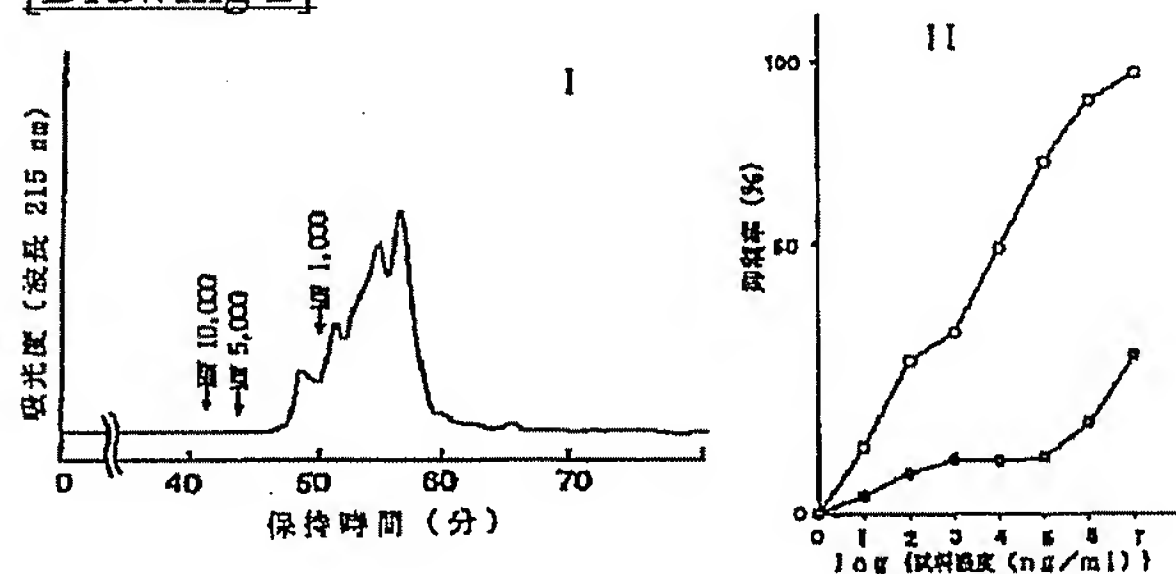
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DRAWINGS

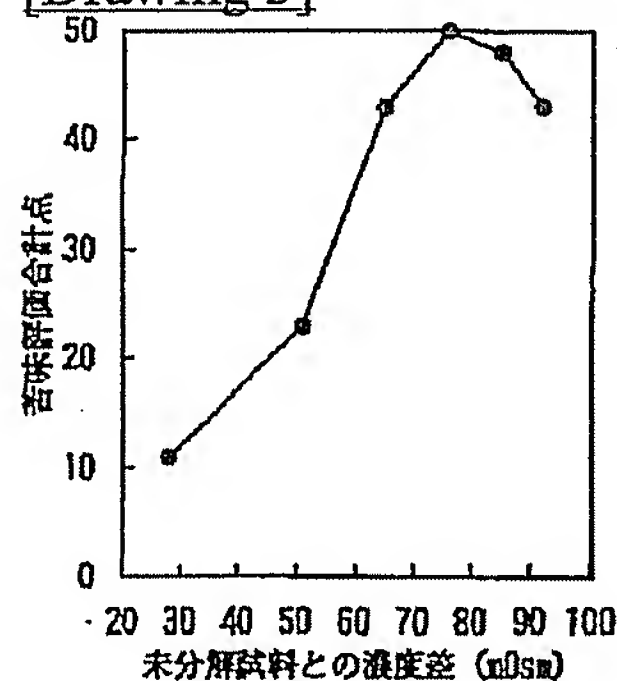
[Drawing 1]



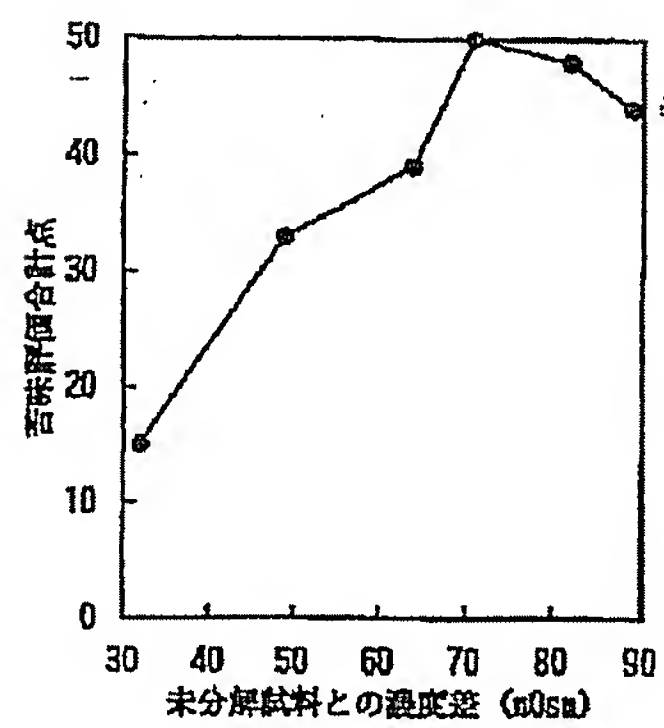
[Drawing 2]



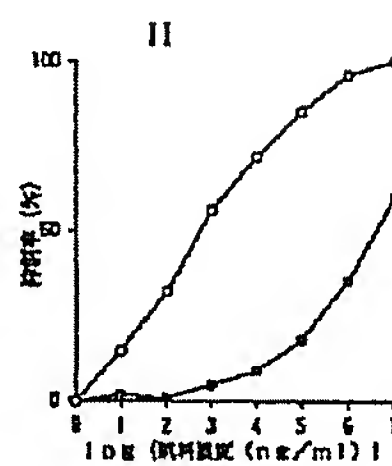
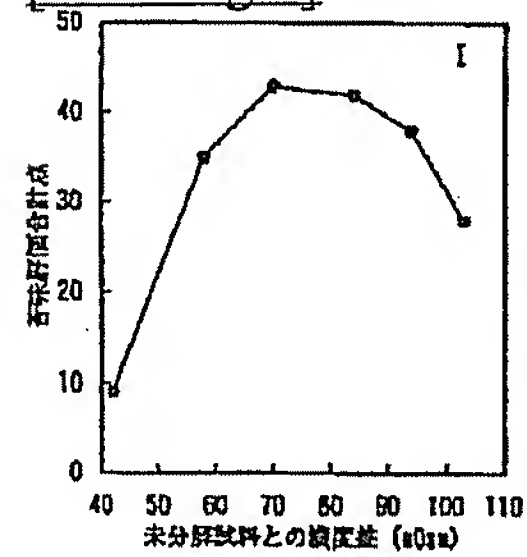
[Drawing 3]



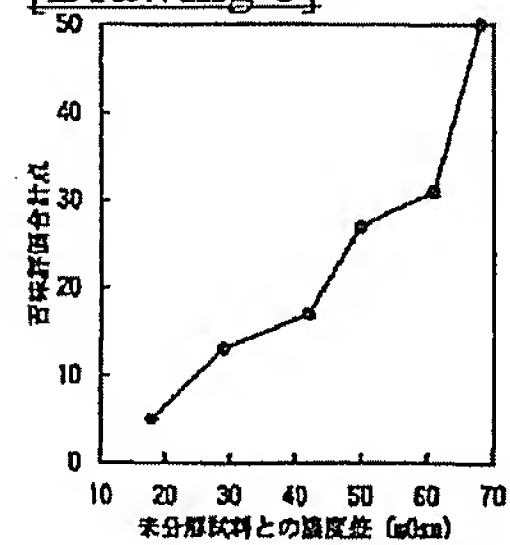
[Drawing 4]



[Drawing 5]



[Drawing 6]



[Translation done.]